PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS: A RANDOMIZED CONTROLLED CLINICAL TRIAL.

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

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ENDORSEMENT BY THE H.O.D. PRINCIPAL/ HEAD OF THE INSTITUTION

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

ABBREVIATIONS

AGEs	Advanced glycation end products
aPDT	Antimicrobial photodynamic therapy
BOP	Bleeding on probing
CAL	Clinical attachment level
DNA	Deoxyribonucleic acid
FMBS	Full mouth bleeding scores
FMPS	Full mouth plaque scores
F.nucleatum	Fusobacterium nucleatum
Ga-Al-As	Gallium-Aluminium-Arsenide
GCF	Gingival crevicular fluid
GI	Gingival index
GR	Gingival recession
GM-CSF	Granulocyte monocyte – colony stimulating factor
HbA1c	Glycated hemoglobin
IL	Interleukin
KTP	Potassium titanyl phosphate
LDD	Local drug delivery
LED	Light emitting diode
LLLT	Low level laser therapy
MB	Methylene blue

mW	Milliwatt
min	Minutes
Nd:YAG	Neodymium doped : Yittrium Aluminium Garnet
nm	Nanometer
PCR	Polymerase chain reaction
PD	Probing depth
PlI / PI	Plaque index
PPD	Probing pocket depth
P.gingivalis	Porphyromonas gingivalis
Pa	Photoablative
PMN	Polymorphonuclear neutrophils
RAL	Relative attachment level
RBC	Red blood cells
ROS	Reactive oxygen species
RT-PCR	Real time polymerase chain reaction
RANKL	Receptor activator of nuclear factor kappa-B ligand
S	Singlet state
S*	Excited singlet state
SFFR	Sulcus fluid flowing rate
s	Seconds
SD	Standard deviation
SRP	Scaling and root planning
T	Excited triplet state

TBO	Toluidine blue
TNF - α	Tumour necrosis factor – α
TGF- β	Transforming growth factor – β
TRAP	Tartrate-resistant acid phosphatase
$^{I}O_2$	Singlet oxygen
OPG	Osteoprotegerin

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Introduction

INTRODUCTION

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss.[1]

Both surgical and nonsurgical periodontal therapy aims at the reduction of bacterial load by eliminating the dental plaque, calculus and root surface irregularities. Systemic antimicrobial therapy may further suppress the periodontal pathogens and augment the effect of conventional mechanical debridement. A major disadvantage of systemic antimicrobial therapy is the insufficient concentration of the drug in the gingival crevicular fluid and also the presence of the microorganisms in a biofilm environment. Furthermore repeated use of antibiotics may lead to the development of resistant microorganisms. So local drug delivery (LDD) evolved as an alternative to systemic antibiotics, however it can be technically difficult at multiple sites.

To overcome most of the problems and complications related to the local and/or systemic use of antibiotics, antimicrobial photodynamic therapy (aPDT) has been suggested as an alternative.[2] aPDT is defined as an oxygen-dependent photochemical reaction that occurs upon light-mediated activation of a photosensitizing compound that leads to generation of cytotoxic reactive oxygen species (ROS), predominantly singlet oxygen, that are toxic to the microorganisms. Major advantages of aPDT are its specificity to the target cells, no collateral damage, initiation of activity only when exposed to light and the lack of development of resistant bacterial species, which is common with the indiscriminate use of antibiotics.[3]

Periodontitis is the 6th complication of diabetes mellitus and diabetes carries a 2 to 3 times higher risk for both the incidence and progression of periodontitis. The pathways of vascular tissue complications in diabetes are mediated through the formation of advanced glycation end products (AGEs) and the increased production of ROS. The interaction between these mechanisms in the periodontium with pre-existing periodontal disease provides insight into the exacerbated periodontal destruction in diabetes and may also explain why diabetic patients are at greater risk for periodontitis.[4]

Uncontrolled diabetes mellitus adversely affects the severity of the periodontal disease and wound healing capacity of the patient and in most instances a nonsurgical approach to periodontal therapy is preferred, with or without the use of appropriate antibiotic therapy. In general, all diabetes mellitus patients should be encouraged to follow a meticulous oral hygiene regimen and to receive supportive periodontal therapy at regular intervals necessary to sustain a high level of periodontal health.[5] aPDT has been used as an adjunct to conventional mechanical therapy and showed promising results, however there are fewer studies in the literature to evaluate the adjunctive effect of aPDT in patients with diabetes mellitus.

In the present study, clinical parameters including plaque index (PI), % of bleeding sites, probing pocket depth (PPD) and clinical attachment level (CAL) were evaluated at baseline and 3 months with an objective to assess the potential of aPDT as an adjunct to scaling and root planing (SRP) in the management of chronic periodontits in type 2 diabetic patients.

Aims & Objectives

AIMS AND OBJECTIVES

- To evaluate the clinical effectiveness of scaling and root planing (SRP) in chronic periodontitis patients with type 2 diabetes mellitus.
- To evaluate the clinical effectiveness of adjunct antimicrobial photodynamic therapy (aPDT) on SRP in chronic periodontitis patients with type 2 diabetes mellitus.
- To compare the clinical outcomes of SRP with or without adjunctive aPDT in patients with chronic periodontitis and type 2 diabetes mellitus testing the hypothesis of adjunctive aPDT being able to improve the outcomes of non- surgical periodontal therapy.

Review of Literature

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) can be defined as the administration of a nontoxic drug or dye known as a photosensitizer either systemically, locally or topically to a patient bearing a lesion (frequently but not always cancer), followed after some time by the illumination of the lesion with visible light (usually long wavelength red light), which, in the presence of oxygen, leads to the generation of cytotoxic species and consequently to cell death and tissue destruction.[6]

COMPONENTS OF PDT

Photodynamic therapy basically involves three nontoxic ingredients: visible harmless light, a nontoxic photosensitizer and oxygen.[7]

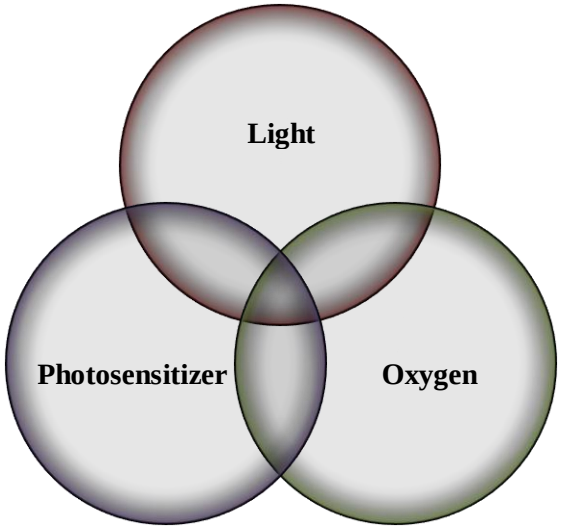


Fig.1 : Components Of PDT

PHOTOSENSITIZER

Photosensitizing agents / photosensitizers are dyes composed of molecules capable of absorbing light energy and using it to promote chemical reactions in cells and tissues when exposed to light.[8]

An ideal photosensitizer must possess photo-physical, chemical and biological properties.

In aPDT, an optimal photosensitizer must have the following properties,

- A high quantum yield of triplet state to obtain large concentrations of the activated drug.
- High singlet oxygen quantum yield
- High binding affinity for microorganisms
- A broad spectrum of action
- Low binding affinity for mammalian cells to avoid the risk of photodestruction of host tissues
- A minimal risk of promoting mutagenic processes
- Low chemical toxicity [9]
- Should be nontoxic and be activated only on illumination.[10]

Range of photosensitizers include,

Grouped into families

Tricyclic dyes with different mesoatoms

- Acridine orange
- Proflavine
- Riboflavin
- Methylene blue
- Fluorescein
- Erythrosine

Tetrapyrroles

- Porphyrins and derivatives
- Chlorophyll
- Phylloerythrin
- Phthalocyanines

Furocoumarins

- Psoralen and its methoxyderivatives
- Xanthotoxin
- Bergaptene [8]

Based on generations

First-generation photosensitizers

- Photofrin derivatives
- Hematoporphyrin derivatives

Second generation photosensitizers

- 5-Aminolevulinic Acid
- Benzoporphyrin Derivative
- Lutetium
- Texaphyrin
- Temoporfin
- Tinethyletiopurpurin
- Talaporfin Sodium [11]

Antimicrobial photosensitizers – phenothiazine dyes

aPDT employs a variety of photosensitizers including toluidine blue O (TBO), methylene blue (MB), erythrosine and hematoporphyrin. TBO and MB have similar physicochemical and chemical properties and are very effective photosensitizers for the inactivation of both gram positive and gram negative bacteria to the treatment.

The relatively porous cytoplasmic membrane of gram positive species permits the entry of photosensitizers into the cell, but an additional outer membrane layer of gram

negative organisms serve as an effective permeability barrier that may reduce or prevent the photosensitizer uptake.

Methylene blue

Methylene blue has been used as a photosensitizer since the 1920s.

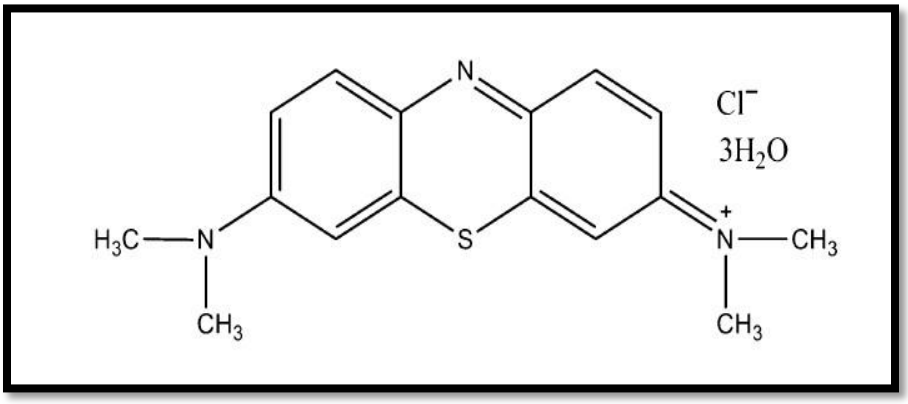


Fig.2 : Structure Of Methylene Blue

Methylene blue undergoes a pronounced cationic charge, can bind to the outer membrane of gram negative organisms and penetrate the bacterial cells with a high degree of selectivity compared to host mammalian cells leading to the destruction of microorganisms.[12]

The hydrophilicity of methylene blue along with its low molecular weight and positive charge permits passage across the porin protein channels in the outer membrane of gram negative bacteria.

Commercially available PDT kits with methylene blue as photosensitizer are,

- Periowave ™
- Helbo ® PDT system. [9]

LIGHT

In PDT, a light source is necessary to activate the photosensitizer.[8] In the past, a variety of light sources such as argon lasers, potassium titanyl phosphate (KTP) and Nd:YAG were used for photosensitizer activation. At present, diode lasers which have a number of advantages like cost effectiveness, portability and user friendliness are used predominantly.[11]

Currently the most commonly applied light sources in PDT are helium neon lasers (633nm), Ga-Al-As diode laser (630-690nm, 830nm or 906nm) and argon lasers (488-415nm). The wavelength of all the above mentioned lasers range from visible light to blue, red and infrared area of some diode laser. A low level exposure produces a high bactericidal effect and hence high level laser energy irradiation is commonly not used to activate the photosensitizer.[12]

For the treatment of larger areas, non coherent light sources such as tungsten filament, quartz halogen, xenon arc and phosphor coated sodium lamps are used.[11] Nonlaser light sources like light emitting diodes (LED) have been used recently owing to their portability, cost effectiveness and light weight properties.[12]

MECHANISM OF ACTION

A molecule of the photosensitizer in its ground singlet state (S) is excited to the singlet state (S*) following absorption of the photon of light and receives the energy of the photon. The S* state molecule does not interact with the surrounding molecules in a significant manner as its lifetime is too short (in nanosecond range). The S* state molecule either emits a photon as light energy (fluorescence) or loses its energy as heat due to internal conversion and may decay back to the original state.

Alternatively, inter-system crossing may occur that involves the change in the spin of an electron and the molecule may convert into an excited triplet state (T). Molecule in the T state has a longer lifetime (microsecond to millisecond range) and can either emit light (phosphorescence) by returning to the ground state or can react following two different pathways.[9]

Type I reaction / Type I photoprocess

In the excited state, the photosensitizer reacts with an organic substrate molecule of the cells and produces free radicals and radical ions by involving in hydrogen-atom abstraction or electron transfer reactions. These highly reactive free radical species interact with endogenous molecular oxygen to produce ROS such as superoxide, hydroxyl radicals and hydrogen peroxide. These ROS are harmful to cell membrane integrity and can cause irreparable biological damage.

Type II reaction / Type II photoprocess

An electronically excited and highly reactive state of oxygen is known as singlet oxygen ($^1O_2$) and it has the ability to interact with a large number of biological substrates owing to its high chemical reactivity. It induces oxidative damage to the bacterial cell by damaging the cell membrane and the cell wall. The T state photosensitizer reacts with oxygen to produce this singlet oxygen.

Singlet oxygen is lethal to various micro-organisms including viruses, bacteria, protozoa and fungi. Due to a short lifetime and very short radius of action, there is limited migration of singlet oxygen from its site of formation. Hence sites of initial cell damage from aPDT are closely related to localization of the photosensitizers making it ideal for application at localized sites without affecting the distant molecules, cells or organs.

Type II photoprocess of aPDT is commonly accepted as the major pathway in microbial cell damage and singlet oxygen is the primary cytotoxic agent which is responsible for the biological effects of the photooxidative process.[12]

The ROS reacts with proteins, organelles, nucleic acids and lipids which are the cellular components and cause irreversible damage by modifying the respiratory chain and increasing the membrane permeability ultimately leading to cell death.[8]

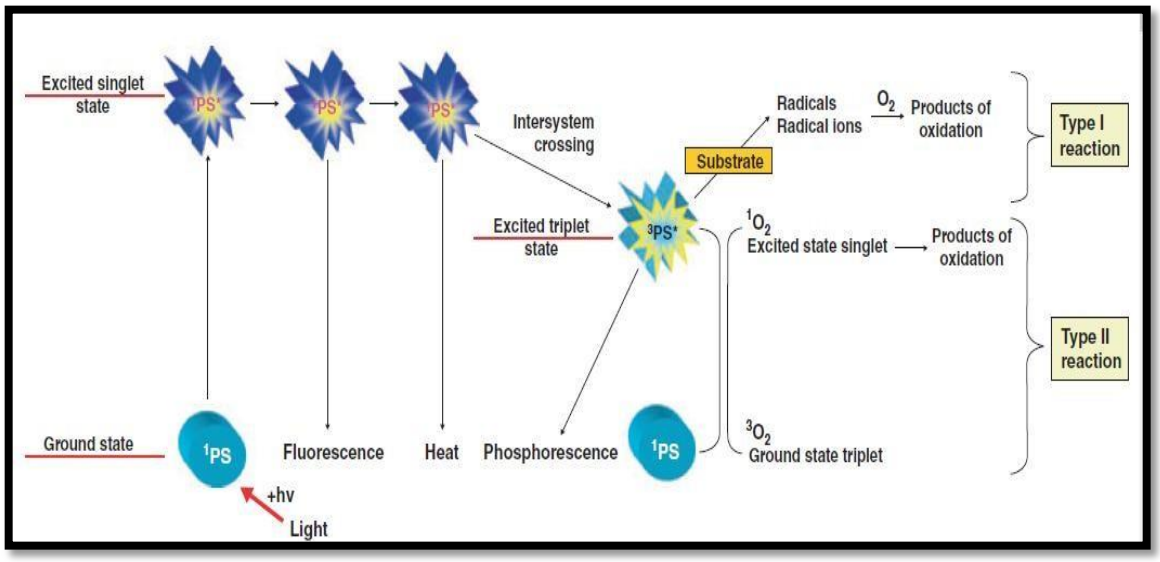


Fig 3 : Mechanism of action - aPDT

ANTIMICROBIAL PHOTODYNAMIC THERAPY IN PERIODONTICS

Biofilms in oral cavity are accountable for two of the most common oral diseases, dental caries and periodontal diseases. The oral cavity harbours various microorganisms including gram positive, gram negative, aerobic and anaerobic bacteria, viruses, fungi, archae and protozoa.[13] Dental plaque is a biofilm i.e it is primarily composed of microorganisms nonrandomly distributed in a shaped matrix or a glycocalyx.[1]

Though conventional mechanical debridement resulted in clinical improvements, it has been demonstrated that mechanical therapy is insufficient for complete removal of all the pathogens due to anatomical complexity of the tooth roots and the ability of the bacteria to invade the soft tissues. Also biofim associated bacteria are less susceptible to antibiotics and repeated antimicrobial therapy may result in resistant organisms. Therefore, there is significant interest in seeking alternative antimicrobial concepts.

In recent times, aPDT has evolved as a treatment modality for localized microbial infections since the free radicals that are formed might be toxic to the bacteria. This therapeutic modality could be an ideal complement to conventional scaling and root planing in periodontal therapy as the polysaccharides in the extracellular matrix of oral biofilms are highly sensitive to ROS and are susceptible to photodamage.[15]

Application of aPDT in periodontal therapy

After a complete debridement of the tooth and root surfaces, the photosensitizer can be placed directly in the periodontal pocket with the help of a blunt cannula or a syringe and needle such that the liquid photosensitizer can easily access the whole root surface. After a resident time it can be activated by the laser light or any visible light through placement of the optical fiber directly in the pocket.[12]

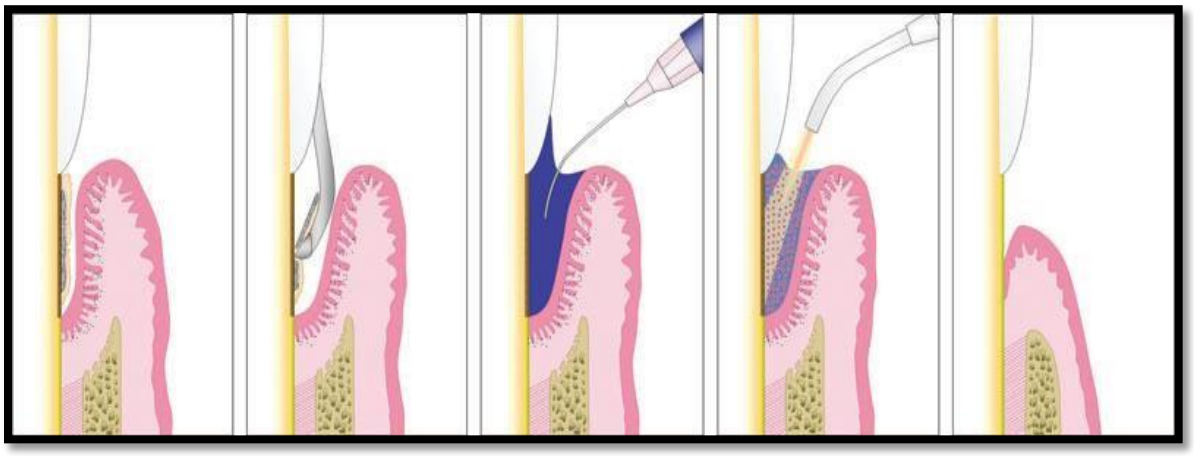


Fig.4 : Application Of aPDT in Periodontal Therapy

Few advantages of PDT in periodontal therapy are,

- Relatively short treatment time
- No need for anaesthesia
- Selective uptake of photosensitizers
- Precise direction of laser light using optical fibers
- Photosensitizers are activated only on illumination thus avoiding damage to adjacent host tissues.
- Repeated applications can be done without the need for total dose limitations
- No resistance to treatment with repeated treatment.

Limitations of PDT

- aPDT requires direction of the light to the appropriate site and tissue depth to be effective.
- aPDT is an ablative procedure and does not yield material for histological diagnosis and hence diagnosis should be made before treatment.[15]

REVIEW OF LITERATURE

Almeida et al (2007)[16] evaluated histologically and radiographically, the effect of photodynamic therapy (PDT) with 100µg/ml methylene blue and 685nm low intensity laser, on the progression of experimentally induced periodontal disease in 120 Wistar rats. The animals were divided into 4 groups: group 1 received no treatment, group 2 was treated topically with methylene blue (MB), group 3 was treated with low-level laser therapy (LLLT) and group 4 was treated with photodynamic therapy (PDT). Rats were sacrificed at days 5, 15 or 30 days postoperatively. Standardized radiographs were taken to measure the bone loss around the mesial root surface of the first molar and histological evaluation of connective tissue, periodontal ligament and alveolar bone was done. The results indicated that radiographic examination showed significantly less bone loss in group PDT compared to control group at 5 and 15 days postoperatively but no significant difference in bone loss at 30 days. At 15 days, the histologic results showed significant differences in the extent of inflammatory reaction in the gingival tissue, with a greater extent of chronic inflammatory reaction in group LLLT. The results suggested that PDT transiently reduced the periodontal tissue destruction.

Almeida et al (2008)[17] histometrically assessed the influence of photodynamic therapy (PDT) on furcation defects in 120 adult male Wistar rats with experimentally induced periodontal disease with 100µg/ml methylene blue and 685nm low intensity laser. The animals were divided into 4 groups: control group (no treatment), methylene blue group (MB), laser group (LLLT) and photodynamic therapy group (PDT). Rats were

sacrificed at 5, 15 or 30 days postoperatively. The area of bone loss in the furcation region of the first molar was histometrically analyzed. PDT group demonstrated less bone loss compared to the other groups at days 7 and 15, the PDT and MB groups demonstrated less bone loss compared to the control and LLLT groups indicating that PDT may be an effective alternative for control of bone loss in furcation areas in periodontitis.

Christodoulides et al (2008)[18] evaluated the clinical and microbiological effectiveness of the adjunctive use of photodynamic therapy (PDT) to nonsurgical periodontal treatment in chronic periodontitis patients receiving initial periodontal therapy. 24 patients with chronic periodontitis were randomly divided into two groups: control group (SRP) and test group (SRP + PDT). The control group received full mouth scaling and root planing (SRP) and the test group received a single episode of PDT with HELBO photodynamic systems and 670nm diode laser as an adjunctive to SRP. Full mouth plaque scores (FMPS), full mouth bleeding scores (FMBS), probing depth (PD), gingival recession and clinical attachment level (CAL) were measured at baseline, 3 and 6 months after initial therapy. Subgingival plaque samples were taken at baseline from the deepest pocket of the quadrant and the same sites were resampled at 3 and 6 months. Microbiologic evaluation of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola, Parvimonas micra, Fusobacterium nucleatum, Campylobacter rectus, Eubacterium nodatum, Eikenella corrodens, and Capnocytophaga spp. was performed at baseline, 3 and 6 months following therapy by using PCR. The results showed no significant difference in CAL, PD, FMPS and microbiologic changes between the groups at 3 and 6 months but

showed a significantly greater improvement in test group with regard to FMBS. The results indicate that additional application of a single episode of PDT to scaling and root planing failed to result in an additional improvement in terms of PD reduction and CAL gain, but it resulted in a significantly higher reduction in bleeding scores compared to scaling and root planing alone.

Braun et al (2008)[19] assessed the effect of adjunctive antimicrobial photodynamic therapy (aPDT) in 20 patients with untreated chronic periodontitis in a split mouth clinical trial. All patients received complete scaling and root planing and using a split mouth design two quadrants were additionally treated with aPDT. aPDT was performed with 660nm diode laser and HELBO photodynamic systems. Sulcus fluid flow rate (SFFR) and bleeding on probing (BOP) were assessed at baseline, 1 week and 3 months after treatment. Relative attachment level (RAL), probing depth (PD) and gingival recession (GR) were evaluated at baseline and 3 months after treatment. The results showed that the values for RAL, PD, SFFR and BOP decreased significantly 3 months after treatment in both the groups with a higher impact on the sites treated with adjunctive aPDT. GR increased 3 months after treatment with and without adjunctive aPDT with no difference between the groups. The results imply that in patients with chronic periodontitis, clinical outcomes of conventional subgingival debridement can be improved by adjunctive aPDT.

Fontana et al (2009)[20] investigated the ability of photodynamic therapy (PDT) to reduce the number of bacteria in biofilms by comparing the photodynamic effects of methylene blue on human dental plaque microorganisms in the planktonic phase and in biofilms. Dental plaque samples were collected from 10 chronic periodontitis patients.

Suspensions of plaque microorganisms from five subjects were sensitized with methylene blue (25 µg/mL) for 5 min and then exposed to 665nm diode laser. Multispecies microbial biofilms developed from the same plaque samples were also exposed to methylene blue (25µg/mL) and irradiated with laser using the same settings. The survival fractions of the microorganisms were calculated by counting the number of colony- forming units. The results connoted that PDT killed approximately 63% of bacteria present in suspension whereas only 32% of the bacteria were killed in the biofilm. The results imply the fact that oral bacteria in biofilms are affected less by PDT than bacteria in the planktonic phase.

Braham et al (2009)[21] developed a model that could simultaneously compare protease inactivation to P. gingivalis killing and in addition, examined the ability of antimicrobial photodynamic therapy (aPDT) with 0.01% methylene blue and 670nm diode laser to neutralize host inflammatory cytokines to determine the potential of this treatment to augment host healing. A 96-well-based, bacterial killing and protease inactivation assay that has the ability to determine the bactericidal effect of aPDT and protease inactivation from the same sample were developed. A pair of identical samples of P. gingivalis inoculums in a photosensitizer formulation in which one of the samples was irradiated for 60 seconds and the other sample was kept in the dark were used. A cytokine inactivation assay that measured E-selectin expression in response to TNF-α and IL-1β was developed to measure the ability of aPDT to inactivate cytokine function. The results showed that a single aPDT treatment in vitro potently inactivated protease activity, reduced the viability of P. gingivalis and also potently and functionally inactivated IL-1β and TNF-α. They concluded that aPDT treatment may augment periodontal treatment by increasing

bacterial killing, inactivating bacterial virulence factors and inactivating host cytokines that impair periodontal restoration.

Al-Zahrani et al (2009)[2] in a single masked randomized controlled trial examined the effects of the adjunctive use of photodynamic therapy (PDT) on the periodontal status and glycemic control of 45 patients with type 2 diabetes mellitus and moderate to severe chronic periodontitis. Patients were randomly assigned to 3 groups : scaling and root planing (SRP) only, SRP plus systemic doxycycline (two × 100mg for day 1 and then 100 mg once a day for 13 days) and SRP plus PDT (0.01% methylene blue and 670nm diode laser). The plaque and bleeding scores, probing depth (PD), clinical attachment level (CAL) and glycosylated hemoglobin (HbA1c) level were recorded at baseline and 3 months after periodontal treatment. There was no significant differences in the mean PD, CAL, plaque deposits, bleeding scores and glucose levels between test and control groups and the results of this study indicated that PDT does not benefit conventional non- surgical periodontal therapy in patients with diabetes.

Polansky et al (2009)[22] designed a randomized-controlled clinical pilot trial to investigate both the clinical effects and the bactericidal potential of PDT applied in conjunction with conventional ultrasonic treatment in 58 patients with chronic periodontitis. Patients with at least three periodontal pockets 5-8mm deep and positivity for Porphyromonas gingivalis were included in this study and were assigned to the PDT or the control group (SRP) based on a randomization list. In each patient, only the four deepest pockets not exceeding 8mm were included in the analysis. All patients received complete supra and subgingival scaling and root planing and the test group was additionally treated with PDT using Helbo PDT system. Clinical parameters including

gingival index (GI), bleeding on probing (BOP), probing pocket depths (PD) and clinical attachment levels (CAL) were recorded at baseline and 3 months after the treatment. Pathogen screening for Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola was conducted at baseline, 10, 42 and 90 days after treatment. The results showed a significant reduction in all the clinical parameters and Porphyromonas gingivalis in both the groups with insignificant intergroup difference. The results imply that application of a single cycle of PDT was not effective as an adjunct to ultrasonic periodontal treatment in patients with chronic periodontitis.

Sigusch et al (2010)[23] evaluated the clinical and microbiological effect of photodynamic therapy (PDT) in Fusobacterium nucleatum–infected patients with chronic periodontitis. 24 patients, in whom only F. nucleatum was detected by baseline polymerase chain reaction (PCR) after SRP, were randomly assigned to PDT and control groups. PDT was carried out once as a full-mouth disinfection with Helbo PDT system in the test group and the control group was treated with the photosensitizer solution (Helbo^{R}), but without laser irradiation. The clinical parameters plaque index (PI), reddening, bleeding on probing (BOP), probing depth (PD), gingival recession, and clinical attachment level (CAL) were recorded at baseline (weeks after SRP), 1, 4, and 12 weeks after PDT. Quantitative analysis of the F. nucleatum DNA concentration was performed by competitive PCR. The results showed significant reductions in reddening, BOP, mean PD and CAL with respect to both the control and the test group. 4 and 12 weeks after PDT, the mean PD and CAL showed significant differences from baseline values and from those of the control group. In the PDT group, 12 weeks after treatment, the F. nucleatum DNA concentration was found to be significantly reduced compared to

the baseline level. The results implied that the adjuvant application of the described PDT method is appropriate to reduce periodontal inflammatory symptoms and to successfully treat infection with F. nucleatum.

Lui et al (2011)[24] designed a single-blinded, split-mouth design clinical trial to evaluate the effects of a combination of photodynamic therapy (PDT) with 1% methylene blue and 940nm low-level laser therapy (LLLT) as an adjunct to nonsurgical treatment in 24 patients with chronic periodontitis. Patients were randomly assigned in a split-mouth design to receive scaling and root planing (SRP) with or without one course of adjunctive PDT and LLLT within 5 days. Plaque scores, bleeding scores, PD and gingival recession (GR) were recorded at baseline, 1 and 3 months after the treatment. Gingival crevicular fluid (GCF) was collected at baseline, 1 week and 1 month to assess IL-1β levels. The results revealed that the test teeth achieved greater reductions in the percentage of sites with bleeding on probing and mean PD at 1 month, significant decrease in GCF volume and a greater reduction of IL-1β levels than the control sites. The results suggest that a combined course of PDT with LLLT could be a beneficial adjunct to nonsurgical treatment of chronic periodontitis on a short-term basis.

Giannelli et al (2012)[25] evaluated the benefits of combination of SRP and sequential photoablative (Pa) and photodynamic (Pd) treatments over SRP alone in 28 patients with chronic periodontitis in a split mouth study. After supragingival scaling, each patient underwent 2 parallel treatments, the teeth on the test maxillary quadrant were treated with laser + SRP, whereas those of the contra-lateral control quadrant were treated with sham-laser + SRP. The gingival mucosa was subjected to Pa treatment with a diode laser operating at 810 nm wavelength. Sham-laser treatment consisted of the same manual

operations performed with the laser switched off. At the end of the Pa or sham treatments, conventional SRP was performed. After 1 week, the laser-irradiated mucosa was subjected to Pd treatment (4-6 weekly applications) with a diode laser operating at 635 nm wavelength and 0.3% methylene blue. PD, CAL and bleeding on probing were recorded at days 0 and 365. Cytofluorescence analysis of gingival exfoliative samples taken at days 0, 15, 30, 45, 60, 75, 90 and 365 was done. The results indicated that the laser + SRP therapy yielded a significant reduction in PD, CAL and BOP as well as in bacterial contamination, especially spirochetes and PMN and RBC shedding in the gingival samples. They concluded that the favourable characteristics of diode lasers used sequentially in photoablative and photodynamic modes as adjuncts to conventional SRP may be considered valuable tools for the treatment of chronic periodontitis.

Berakdar et al (2012)[26] examined the added efficacy of photodynamic therapy (PDT) on scaling and root planing (SRP) in 22 patients with chronic periodontitis in a split mouth study. In each patient 2 teeth belonged to the control group (SRP) and 2 to the test group (SRP + PDT). After SRP, teeth belonging to the test group received PDT (with 0.005% methylene blue and 670nm diode laser). Bleeding on probing (BOP), plaque index (PlI) probing depth (PD) and clinical attachment level (CAL) were assessed at baseline (1week before therapy), 1, 3 and 6 months after the therapy. The results showed a greater reduction of the PD was achieved by a combination of SRP/PDT, and it was statistically significant after 6 months. The results demonstrated SRP in combination with PDT to be effective than SRP alone and is therefore suitable as an adjuvant therapy to the mechanical debridement of the periodontal pockets in patients with chronic periodontal diseases.

Dilsiz et al (2013)[27] designed a randomized controlled clinical trial to compare the clinical effects of Potassium–Titanyl– Phosphate Laser (KTP) and photodynamic therapy (PDT) on the outcomes of treatment of chronic periodontitis. According to a split-mouth design, each patient was treated with 3 different modalities, group A - SRP alone, group B - SRP + PDT (1% methylene blue and 808nm diode laser) and group C - SRP + KTP (532nm) laser. Plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) were recorded at baseline and at 6 months after therapy. Results showed that all treatments yielded significant improvements in terms of BOP, PD decrease and CAL gain compared to baseline values but group C showed a greater reduction in PD and gain in CAL compared to the other groups. It was concluded that in patients with chronic periodontitis, clinical outcomes of conventional periodontal treatment of deeper pockets can be improved by using adjunctive KTP laser and single application of photodynamic therapy was not effective as an adjunct to traditional SRP.

Balata et al (2013)[28] designed a randomized controlled clinical trial to evaluate the effects of photodynamic therapy (PDT) as an adjunct to full-mouth ultrasonic debridement in the treatment of severe chronic periodontitis. 22 patients were randomly assigned by a split mouth design to one of the treatments: SRP with PDT (test group) or SRP without PDT (control group). All patients received full-mouth ultrasonic debridement and PDT was performed on only one side of the mouth with 0.005% methylene blue as photosensitizer and 660nm low power laser. Plaque index (PI), gingival index (GI), bleeding on probing (BOP), gingival recession (GR), probing depth (PD) and clinical attachment level (CAL) were recorded at baseline, 1, 3 and 6 months

after treatment. The results showed an improvement in BOP, PD and CAL after treatment, in both groups, but without any difference between them and implied that the PDT did not provide any additional benefit to those obtained with full-mouth ultrasonic debridement alone.

Luchesi et al (2013)[29] investigated the clinical, microbiological and immunological effects of photodynamic therapy (PDT) as an adjunct to scaling and root planing (SRP) in class II furcation sites in a double-blinded, parallel, randomized controlled clinical trial. 42 subjects were randomly allocated to a test (PDT) or control group (non-activated laser/ only photosensitizer). After phase I therapy the sites presenting class II furcation lesions were randomly assigned to receive PDT (10gm/ml methylene blue and 660nm laser) or non-activated laser/only photosensitizer. Plaque scores, bleeding scores, gingival margin (GM), probing depth (PD) and clinical attachment level (CAL) were recorded at baseline, 3 and 6 months after the treatment. GCF samples were collected at baseline, 3 & 6 months and microbiological analysis was done with RT-PCR and cytokine profile was analyzed using multiplexed bead immunoassay. The results showed that clinical parameters improved after both therapies with no differences between the groups at any time point while RT-PCR showed a decrease in Porphyromonas gingivalis and Tannerella forsythia only in the PDT group at 6 months. Regarding cytokines, IL-4 and IL-10 levels increased in both groups at 6 months and GM-CSF, IL-8, IL-1b and IL-6 levels decreased only in the PDT group after 3 months. They concluded that PDT did not promote clinical benefits for class II furcations, however advantages in local levels of cytokines and a reduction in periodontopathogens were demonstrated.

Betsy et al (2014)[30] in a single-centred randomized controlled clinical trial involving 90 patients evaluated whether adjunctive use of aPDT to SRP has any short-term effectiveness in the management of patients with chronic periodontitis in terms of clinical parameters and halitosis. Each participant was randomly assigned either to SRP (control) or SRP + aPDT (test) groups. All patients received complete supragingival and subgingival scaling and root planing and the test group was managed by aPDT (with 10mg/ml methylene blue and 655nm diode laser) in addition to SRP. Plaque index, gingival index, bleeding index, PD and CAL were recorded at baseline, 2weeks, 1, 3 and 6 months after the treatment. Halitosis as perceived by the patient was assessed by hand on mouth technique at baseline, 1, 3 and 6 months of treatment. The results showed that PD and CAL showed statistically significant reduction in the test group on evaluation at 3 months and 6 months and a significant difference was detected for the test group at 1 month in terms of halitosis as compared to the control group. The results imply that aPDT has an important role to play in improving clinical outcomes obtained through SRP and it would be worthwhile to repeat aPDT at frequent intervals to obtain a more definitive cure.

Kolbe et al (2014)[31] designed a split-mouth, randomized controlled trial to investigate the clinical, microbiologic, immunoinflammatory and patient-centered effects of PDT as a monotherapy during periodontal maintenance in 22 patients with atleast 3 residual pockets. The experimental sites presenting residual pockets were randomly assigned to receive PDT (with 10mg/ml methylene blue and 660nm diode laser), PS (photosensitizer
- 10mg/ml methylene blue) or SRP, all as exclusive treatment. Plaque index, gingival index, bleeding on probing, position of gingival margin, PD and CAL were recorded at

baseline, 3 and 6 months after the treatment. GCF samples were collected at baseline, 3 & 6 months and microbiological analysis was done with RT-PCR and cytokine profile was analyzed using multiplexed bead immunoassay. The results reported lower levels and an inferior frequency detection of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis respectively in the PDT protocol and only patients in the PDT protocol exhibited augmented levels of anti-inflammatory interleukin IL-4 and reduced proinflammatory IL-1β and IL-6 throughout the study. They concluded that PDT as an exclusive therapy may be considered a non-invasive alternative for treating residual pockets, offering advantages in the modulation of cytokines.

Garcia et al (2014)[32] assessed the bone loss and the immune inflammatory response of 126 adult male Wistar rats to 2 photosensitizing agents (MB and TBO) at 2 different concentrations in antimicrobial photodynamic therapy (aPDT), used as an adjuvant therapy in the treatment of periodontitis. Experimental periodontitis was induced in the mandibular first molars and the animals were divided into nine groups: G1 – scaling and root planing (SRP), G2 – SRP + 100 µg/mL of methylene blue (MB), G3 – SRP + 10 mg/mL of MB, G4 – SRP + 100 µg/mL of toluidine blue (TBO), G5 – SRP + 10 mg/mL of TBO, G6 – SRP + 100 µg/mL of MB and laser, G7 – SRP + 10 mg/mL of MB and laser, G8 – SRP + 100 µg/mL of TBO and laser and G9 – SRP + 10 mg/mL of TBO and laser. Bone loss (BL) in the furcation region was evaluated using histomorphometric analysis and immunohistochemical analyses were done to detect the receptor activator of nuclear factor-kappa B ligand (RANKL), osteoprotegerin (OPG) and tartrate-resistant acid phosphatase (TRAP). The results showed that there was significantly less BL in animals treated with aPDT using low concentrations of MB and TBO at 7, 15 and 30

days and immunohistochemical analysis revealed decreased RANKL and increased OPG in the aPDT groups and decreased TRAP-positive cells in G6 and G8 and concluded that aPDT, using low concentrations of MB and TBO, was the most effective adjuvant therapy to SRP, acting indirectly as a downregulator of the molecular mechanisms that control bone resorption in periodontitis.

Carvalho et al (2015)[33] in a randomized controlled clinical trial, evaluated the clinical and microbiological effects of PDT in the treatment of residual pockets of 34 patients with chronic periodontitis subjected to supportive therapy. 34 subjects presenting at least four sites with residual pockets were randomly assigned to test – PDT (with 0.01% methylene blue and 660nm diode laser) or control (sham procedure) group. The treatment was repeated 3, 6 and 9 months after the initial procedure. Clinical parameters such as probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BoP) and plaque index (PI) were measured before intervention and after 3, 6 and 12 months. Subgingival samples were collected at baseline, 7 days, 3, 6 and 12 months. Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia were quantified by RT-PCR. The results showed that all treatments resulted in significant clinical improvement in patients with residual periodontal pockets but did not find any additional significant benefit of PDT in terms of PPD, CAL, BOP and pathogens levels reduction and concluded that the PDT protocol used in this study is not superior to supragingival plaque control in persistent pockets.

Ramos et al (2016)[34] designed a double-blinded, placebo-controlled clinical study to compare, clinically and immunologically, a protocol of multiple antimicrobial photodynamic therapy (aPDT) applications and the use of systemic doxycycline as

adjuvant to SRP on the treatment of uncontrolled type 2 diabetic patients. 30 patients with Hba1c >7% were randomly assigned to two groups, SRP + aPDT and SRP + Doxy. All patients received complete supra and subgingival scaling and root planing. Patients in SRP+aPDT group received PDT applications with Helbo PDT system on 0, 3, 7, and 14 days post therapy with a placebo capsule. Patients in SRP+doxy received systemic doxycycline 100 mg/day for 14 days with a first dose of 200 mg and the same protocol used on SRP + aPDT group, without light exposure. Plaque score (PS), bleeding on probing, probing depth, suppuration, gingival recession, and clinical attachment level, percentage of pockets with desired clinical endpoint were measured at baseline and 3 months after therapy. Cytokine profile was assessed at 0, 1 and 3 month to measure IL- 1β, TNF-α and TGF-β in gingival crevicular fluid. The results showed no significant difference in HbA1c, between treatments and the SRP + aPDT group showed advantage on reducing moderate pockets in single- rooted teeth and IL-1β levels at 3 months. There were no significant differences between TNF-α and TGF-β. They concluded that both treatments were able to improve the periodontal treatment outcomes in uncontrolled type 2 diabetic patients and djunctive use of aPDT apparently presents advantages towards systemic doxycycline when dealing with moderate PPD in single- rooted teeth.

Materials and Methods

MATERIALS AND METHODS

PATIENT SELECTION

The study sample included 24 type 2 diabetic patients with chronic periodontitis, selected from the outpatient ward, Department of Periodontology, K.S.R Institute of Dental Science and Research, Tiruchengode, Namakkal district, Tamil Nadu. Study protocol was explained to the patients and written informed consent and institutional ethical clearance was obtained.

INCLUSION CRITERIA

- Patients between 30-60 years of age (male and female)
- Patients with type 2 diabetes mellitus with presence of at least two teeth with a probing depth of ≥ 5mm and clinical attachment loss of ≥3mm in each quadrant with the presence of at least 16 remaining teeth with a minimum of four teeth in each quadrant.
- Lack of furcation involvement and mobility.

EXCLUSION CRITERIA

- Pregnant and lactating women
- Patients with systemic diseases excluding type 2 diabetes mellitus
- Periodontal treatment within the past 12 months
- Use of systemic antibiotics within the last 6 months
- Smokers and pan chewers

STUDY DESIGN

24 type 2 diabetic patients with chronic periodontitis were selected for this study. In each patient, one quadrant of the maxillary arch was selected as test and the other quadrant was selected as the control randomly. The treatment protocol included scaling and root planing for the control group and scaling and root planing with an adjunct antimicrobial photodynamic therapy with 1% methylene blue as photosensitizer and 980nm diode laser for the test group.

Armamentarium for in vivo examination:

- Mouth mirror
- William's graduated periodontal probe
- Dental explorer
- Cotton pliers
- Cotton roll
- Stainless steel tray
- Gloves
- Face mask

CLINICAL PARAMETERS:

1. Plaque Index - Loe's modification (1967)[35]
2. Gingival Index - Loe's modification (1967)[35]
3. Probing pocket depth[1]
4. Clinical attachment level[1]

PLAQUE INDEX (PlI)

The Plaque Index was described by Silness J and Loe H. in 1964 and modified by Loe H. in 1967. The plaque was assessed using a mouth mirror and dental explorer after air-drying the teeth to assess plaque on the different areas namely mesiofacial, facial, distofacial and lingual surfaces.

Instruments used:

Mouth mirror and a dental explorer.

Airdrying of the teeth and gingiva.

The teeth were airdried and examined visually. When no plaque was visible an explorer was used on the surface. The explorer was passed across the surface in the cervical third and near the entrance to the gingival sulcus. The following scores were given.

Scores for Plaque Index

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only by running a probe, across the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and /or on the gingival margin and adjacent tooth surface.

Calculation of PlI:

PlI score for the area:

Each area (disto-facial, facial, mesio-facial, lingual) is assigned a score from 0 to 3.

PlI score for a tooth:

The scores from the four areas of the tooth are added and then divided by four.

PlI score for the individual:

The indices for each of the teeth are added and then divided by the total number of teeth examined. The scores range from 0 to 3.

Interpretation:

Excellent	'0'
Good	0.1 – 0.9
Fair	1.0 – 1.9
Poor	2.0 – 3.0

GINGIVAL INDEX (GI)

The Gingival Index (GI) was developed by Loe H. and Silness J. in 1963, solely for the purpose of assessing the severity of gingivitis and its location in four possible areas by examining only the qualitative changes (i.e., severity of the lesion) of the gingival soft tissue. In 1967, Loe detailed the sequence of examination to include entire teeth instead of index teeth.

Instruments used:

Mouth mirror and a periodontal probe.

The tissues surrounding each tooth were divided into four gingival scoring units: distofacial papilla, facial margin, mesio-facial papilla and the entire lingual gingival margin. The teeth and gingiva should be dried lightly with a blast of air and /or cotton rolls. Each of the 4 gingival units were assessed and following scores were given.

Scores for Gingival Index

Score	Criteria
0	Absence of inflammation/normal gingiva.
1	Mild inflammation, slight change in color, slight edema; no bleeding on probing.
2	Moderate inflammation; moderate glazing, redness, edema and hypertrophy, bleeding on probing.
3	Severe inflammation; marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding.

Calculation of GI:

GI Score for the area:

Each area (disto-facial, facial, mesio-facial, lingual) is assigned a score from 0 to 3.

GI Score for a tooth:

The scores from the four areas of the tooth are added and then divided by four.

GI score for the individual:

The indices for each of the teeth are added and then divided by the total number of teeth examined. The scores range from 0 to 3.

Interpretation

Gingival scores	Condition
0.1 – 1.0	Mild Gingivitis
1.1 – 2.0	Moderate Gingivitis
2.1 – 3.0	Severe Gingivitis

Probing pocket depth (PPD) and Clinical attachment level (CAL):

Impressions of the upper and lower teeth were taken to fabricate customized splints adapting to the teeth by friction fit. These splints were used to assure reproducible measuring points for both PPDs and CAL. Therefore, the individual splints (thermoplastic resin – Bioart – Brazil) were fabricated for every subject by a vacuum- forming process. The oral and the facial surfaces of the material were trimmed and for every site under study, a groove was made on the splint to position the calibrated periodontal probe and to facilitate a reproducible probe position during the measurements.

Probing pocket depth was measured as the distance between the free gingival margin and the base of the pocket and clinical attachment level was measured as the distance between the cementoenamel junction and the base of the pocket.

PREPARATION OF THE PHOTOSENSITIZER SOLUTION

Armamentarium

- Pure methylene blue (powder form)
- Distilled water
- Borosil 500 ml beaker & cylinder
- Whatman Filter Paper No 1
- Stirrer
- Amber bottle
- Electronic weighing machine

Formulation

1% methylene blue

5mg of methylene blue powder (Sigma Aldrich) was added to 500 ml distilled water in a borosil beaker and stirred with stirrer until it dissolved completely. The mixture was filtered twice through Whatman filter paper to remove any particles and remnants. The resulting solution consisted of 500 ml of 1% methylene blue. The solution was transferred to an amber bottle to prevent any photoreactivity and it was stored and used for the study.

TREATMENT PROTOCOL

Armamentarium

- Latex gloves
- Face mask
- Mouth mirror
- Dental explorer
- Cotton pliers
- William's graduated periodontal probe
- Sterile cotton pellets and gauze pieces
- Stainless steel trays
- Topical anaesthesia (Lignocaine gel)
- Piezoelectric scaler tips (EMS)
- Universal curettes (2R/2L & 4R/4L)
- Dappen dish

- 1% methylene blue (photosensitizer)
- 31 gauge needle with syringe
- Saline
- 980nm diode laser (ZOLAR - photon plus)
- Safety glasses

Treatment procedure

All patients received periodontal treatment comprising SRP of all periodontally involved teeth employing both hand instruments (universal curettes – (2R/2L & 4R/4L) and a piezoelectric ultrasonic handpiece (EMS). Topical anesthesia was used whenever required. Using a split-mouth design, test quadrant was additionally treated with aPDT. Therefore, after periodontal debridement, the quadrants were assigned to different groups randomly by throwing a dice. aPDT was performed with a diode laser (wavelength: 980 nm, output power: 100 mW, Zolar photon plus) in combination with a photosensitizer dye (methylene blue – Sigma Aldrich). Periodontal pockets were rinsed with 1% methylene blue using 31gauge needle starting from the bottom of the pocket to achieve both a complete filling of the pocket and coating of the root surface. After 5 min. residence time, the pockets were rinsed with sterile saline to remove excess photosensitizer, which could act as an optical shield. Employing the laser probe, the remaining photosensitizer was activated for 10 s per site (60s/1min per tooth), 100mW power, 980nm wavelength and continuous mode. The application time of both the photosensitizer and laser light was monitored by a time-controller. Clinical measurements were recorded at baseline and 3 months postoperatively.

FIGURE 5 : PREPARATION OF 1% METHYLENE BLUE

A. Commercially available methylene blue

B. 5g of methylene blue

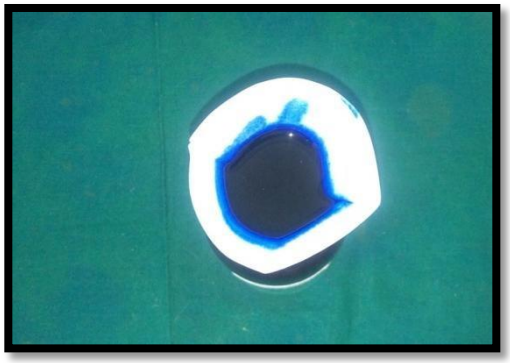
C. 500ml of distilled water

D. Methylene blue added to distilled water

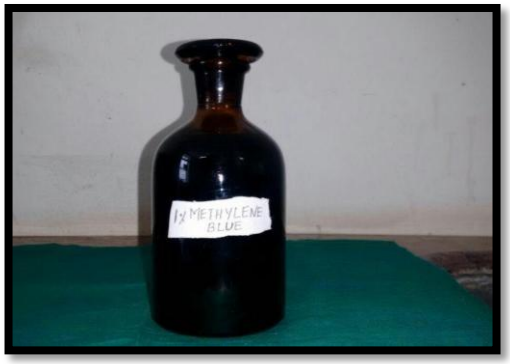
E. Stirred with a stirrer

F. Whatman filter paper

G. Filtration process



H. Filtration process



I. 1% methylene blue

FIGURE 6 : FABRICATION OF STENT

A.Thermoplastic Resin

B. Vacuum forming machine – front view

C. Vacuum forming machine – lateral view

D. High power vacuum suction

E. Prepared stent

F. Trimmed and ready for use

FIGURE 7 : ARMAMENTARIUM FOR CLINICAL PROCEDURE

A. Armamentarium for non surgical therapy and 1% methylene blue

B. Armamentarium for diode laser

FIGURE 8 : PREOPERATIVE VIEW

A. Front view

B. Left lateral view

C. Right lateral view

D.Lingual view

E.Probing depth – SRP side

F. Probing depth – SRP + aPDT side

FIGURE 9 : INTRAOPERATIVE

A.A pplication of photosensitizer

B.Irradiation with laser

FIGURE 10 : POSTOPERATIVE VIEW

A. Front view

C. Right lateral view

B. Left lateral view

D.Probing depth – SRP + aPDT side

E. Probing depth – SRP side

Statistical Analysis

STATISTICAL ANALYSIS

PAIRED t TEST:

Paired t test is applied when there is a pair of data from single element in an observation. Data are collected before and after the intervention, so that the same group acts as both case and control. Then the mean of both the groups are compared to get the t value.

P VALUE:

The P value or calculated probability is the estimated probability of rejecting the null hypothesis of a study question when that hypothesis is true. Differences between the two populations were considered significant when $p < 0.05$.

Results

RESULTS

A total of 6 females and 12 males were enrolled in the study, and all the patients were in the age group 35 to 60years. The intervention was done in the maxillary arch. In each patient, test sites were selected randomly and treated with SRP + aPDT and control sites were treated with SRP alone.

Table no. 1 shows the age and sex distribution of chronic periodontitis patients with type 2 diabetes mellitus All the patients belong to the age group 35 - 60 years, with 50% of them in the age group of 40 – 50 years and 67% of them being males.

Table no.2 shows the mean and standard deviation of the plaque index of chronic periodontitis patients with Type 2 diabetes mellitus before and after the interventions. The mean PI has been 1.33 and 0.56 before and after interventions respectively. Paired t-test has been used to compare the two mean values. The significant p-value infers that both the interventions are effective in reducing the plaque index of the chronic periodontitis patients with type 2 diabetes mellitus.

Table no.3 shows the mean and standard deviation of the clinical variables before and after SRP and its effectiveness. The second objective of the study is to find out effectiveness of the SRP in reducing the clinical variables of the chronic periodontitis patients with type 2 diabetes mellitus.

Before intervention the mean percentage of bleeding sites has been 94.5 and after the intervention the mean percentage of bleeding sites has reduced to 21.5. The Paired t-test has been used to find out whether the reduction in the mean bleeding sites is

statistically significant or not. The significant p-value infers that SRP has been effective in reducing the percentage of bleeding sites.

Similarly the other two clinical variables probing pocket depth and clinical attachment level has been compared. Before intervention the mean probing pocket depth has been 5.44mm and after the intervention the mean probing pocket depth has reduced to 3.66mm. Before intervention the mean clinical attachment level has been 5.54mm and after the intervention the mean clinical attachment level has been reduced to 3.68mm. The significant p-value of the two variables implies that SRP has been effective in reducing the probing pocket depth and clinical attachment level of chronic periodontitis patients with type 2 diabetes mellitus.

Table no.4 shows the mean and standard deviation of the clinical variables before and after SRP + aPDT and its effectiveness. One of the objectives of this study is to find out the effectiveness of the SRP + aPDT intervention. Before intervention the mean percentage of bleeding sites has been 95.2 and after the intervention the mean percentage of bleeding sites has been reduced to 13.5. The Paired t-test has been used to find out whether the reduction in the mean bleeding sites is statistically significant or not. The significant p-value infers that SRP + aPDT has been effective in reducing the percentage of bleeding sites.

Similarly the other two clinical variables probing pocket depth and clinical attachment level has been compared. Before intervention the mean probing pocket depth has been 5.43mm and after the intervention the mean probing pocket depth has been reduced to 3.42mm. Before intervention the mean clinical attachment level has been 5.36mm and after the intervention the mean clinical attachment level has

reduced to 3.34mm. The significant p-value of the two variables indicates that SRP + aPDT has been effective in reducing the probing pocket depth and clinical attachment level of chronic periodontitis patients with type 2 diabetes mellitus.

Table no.5 shows the mean and standard deviation of the intervention wise difference between the clinical variables before and after the interventions. The main objective of the study has been to find out whether any significant variation exists between the SRP + aPDT and SRP alone.

The mean difference in the percentage of the bleeding sites before and after intervention has been 81.7 and 72.8 for the SRP + aPDT and SRP respectively. The significant p-value infers that the mean percentage of the bleeding sites is statistically different for the two interventions. Further it indicates that SRP + aPDT intervention has been better than SRP alone in reducing the percentage of bleeding sites. The mean values are also shown in graph no.1

The difference in the mean probing pocket depth before and after the intervention has been 2.0 mm and 1.76mm for the SRP + aPDT and SRP respectively. The non-significant p-value infers that the outcome has been similar for the two interventions. Both the interventions are equally effective in reducing the probing pocket depth in chronic periodontitis patients with type 2 diabetes mellitus. The mean and standard deviation values are also shown in graph no.2.

The difference in the mean clinical attachment level before and after the intervention has been 2.02mm and 1.86mm for the SRP + aPDT and SRP alone respectively. The non-significant p-value infers that both the interventions are equally

effective in reducing the clinical attachment level of the chronic periodontitis patients. The mean and standard deviation values are also shown in graph no. 2.

Table no. 1 : Age and sex distribution of chronic periodontitis patients with type 2 diabetes mellitus

Variable	No.	%
Age		
30 – 40 years	4	22
40 – 50 years	9	50
50 – 60 years	5	28
Sex		
Male	12	67
Female	6	33

Table no.2 : Comparison of mean plaque index before and after the interventions

Plaque Index	Mean	SD	Paired t-test	df	P-value
Before	1.333	0.2787	10.760	17	.000
After	0.561	0.1720			

Table no.3: Effectiveness of SRP in chronic periodontitis patients with Type 2 diabetes mellitus

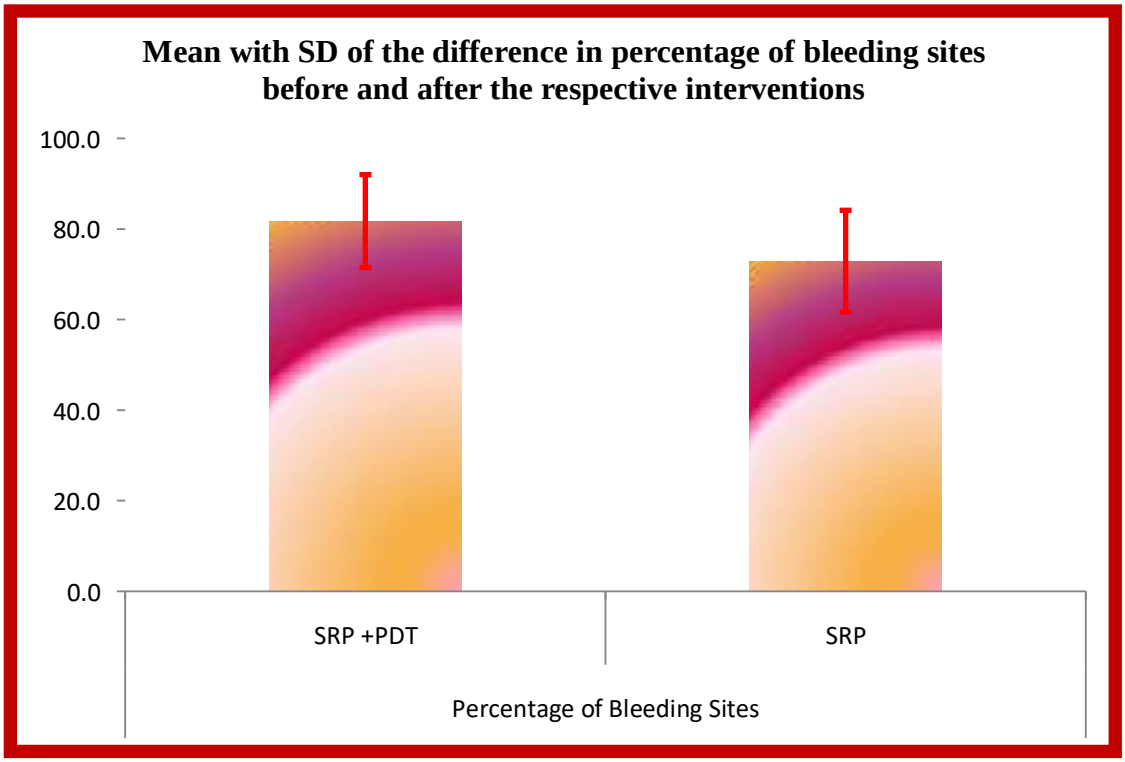
Variable	Assessment	Mean	SD	Paired t-test	df	P-value
% of Bleeding Sites	Before	94.444	9.4882	27.551	17	.000
	After	21.556	7.2292			
PPD	Before	5.4250	0.77094	15.086	17	.000
	After	3.6639	0.77919			
CAL	Before	5.5400	0.84567	17.552	17	.000
	After	3.6767	0.78582			

Table no.4: Effectiveness of SRP + Photodynamic therapy in chronic periodontitis patients with Type 2 diabetes mellitus

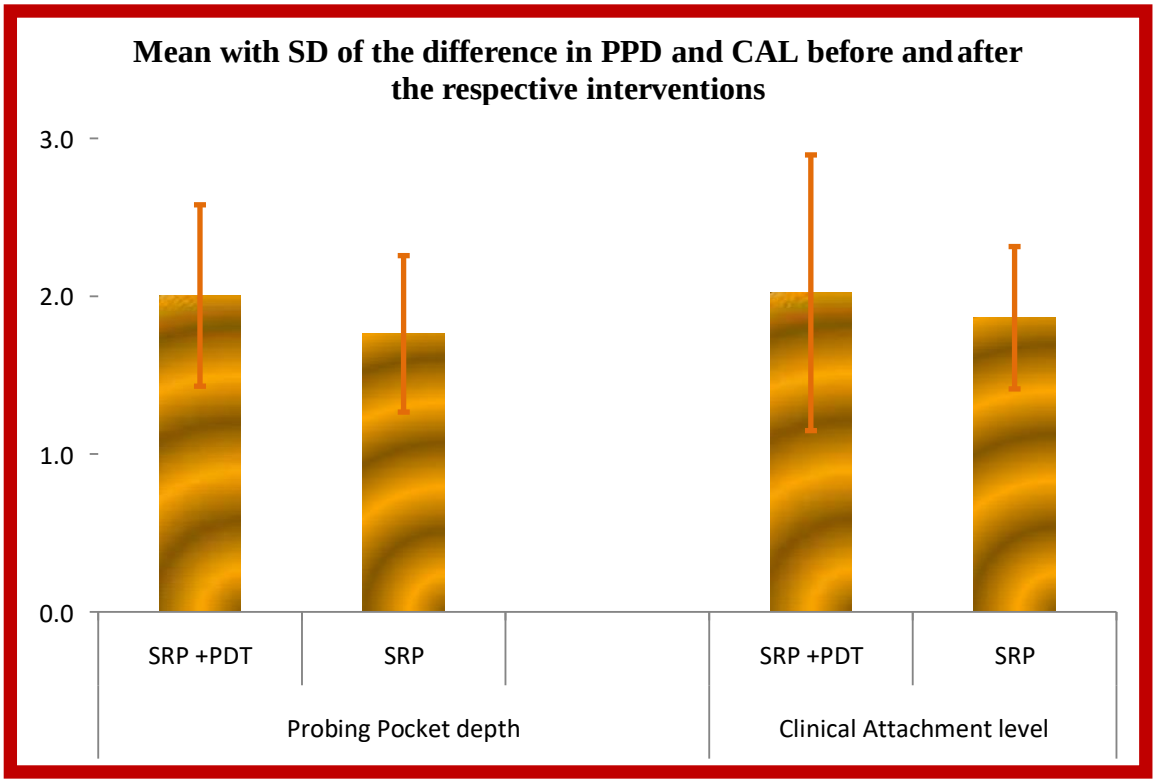
Variable	Assessment	Mean	SD	Paired t-test	Df	P-value
% bleeding sites	Before	95.222	8.4196	33.868	17	.000
	After	13.500	5.2608			
PPD	Before	5.4261	0.81582	14.804	17	.000
	After	3.4222	0.73860			
CAL	Before	5.3600	0.94582	9.822	17	.000
	After	3.3389	0.63075			

Table no.5: Comparison between the effectiveness of SRP + aPDT and the effectiveness of SRP

Variable	Intervention	Difference between before and after intervention		Paired t-test	df	P-value
		Mean	SD			
% of bleeding sites	SRP + PDT	81.7222	10.23722	5.847	17	.000
	SRP	72.8889	11.22439			
PPD	SRP +PDT	2.0039	0.57428	1.509	17	0.150
	SRP	1.7611	0.49529			
CAL	SRP +PDT	2.0211	0.87305	.924	17	0.369
	SRP	1.8633	0.45041			



Graph 1 : Mean with SD of the difference in % of bleeding sites before and after respective interventions



Graph 2 : Mean with SD of the difference of PPD and CAL of the chronic periodontitis patients before and after the respective interventions.

Discussion

DISCUSSION

Chronic periodontitis is an infectious disease resulting in inflammation of the supporting tissues of the teeth, progressive attachment loss and bone loss. Plaque accumulation on the tooth and the gingival surface at the dentogingival junction is considered the primary initiating agent in the etiology of chronic periodontitis.[1]

The association between diabetes mellitus and periodontitis is widely accepted. Epidemiologic studies demonstrate an increase in infection susceptibility, especially to periodontal diseases in patients with uncontrolled diabetes or poor glycemic control. There is also rapid progression and increased severity of periodontal diseases in diabetic patients.[3] In most of these instances a nonsurgical approach is preferred.[5]

The principal objective of nonsurgical periodontal therapy is to reinstitute the gingival health by complete removal of elements like biofilm, calculus and endotoxins from the tooth surface. The nonsurgical therapy by means of scaling and root planing helps in the removal of supragingival and subgingival plaque, calculus and necrotic cementum.[1] But SRP might not lead to total eradication of the microbes which are situated deep within the periodontal tissues, dentinal tubules and other inaccessible areas owing to anatomical complications.[12] Therefore other treatment options like antimicrobial PDT has been considered as adjuncts to SRP.

aPDT is effectuated by singlet oxygen, which has a direct effect on the polysaccharides present in the extracellular matrix of a bacterial biofilm which is receptive to photodamage and resistance development to the cytotoxic action of singlet oxygen or free radicals seem to be implausible. aPDT is effective against antibiotic

resistant and antibiotic susceptible bacteria equally and repeated photosensitization has not lead to the development of resistant strains.[19]

Antimicrobial effect of PDT has been assessed in various in vitro studies, animal studies and in vivo studies by Pfitzner et al (2004)[36], Hayek et al (2005)[37], Qin et al (2008)[38], Christodoulides et al (2008)[18], Fontana et al (2009)[20], Braham et al (2009)[21], Sigusch et al (2010)[23], Nagahara et al (2013)[39], Chui et al (2013)[40], Moreira et al (2015)[41] and Decker et al (2016)[42] with different photosensitizers, wavelengths of light and resident times and have proven to be successful.

The aim of the present study was to evaluate the potential of antimicrobial photodynamic therapy (aPDT) as an adjunct to scaling and root planing in the treatment of chronic periodontitis patients with type II diabetes mellitus. 12 males and 6 females with chronic periodontitis and type II diabetes mellitus have been treated in a split mouth design for this study. All patients received full mouth scaling and root planing and the test sites received a single application of photodynamic therapy with 1% methylene blue as photosensitizer and 980nm diode laser as light source.

In the present study, it can be justified to employ a split-mouth design, as the photosensitizer alone is not capable of generating an antimicrobial effect and the PDT protocol requires the photosensitizer dye to be activated by laser. As only the test quadrants were irradiated by laser light, an effect on bacteria in the control quadrants was not possible, even if some dye should have accidentally come in contact with the tissues of the control quadrants. Also high power suction was continuosly used throughout the procedure.

In this study, a 3 month evaluation period has been chosen, as the periodontal pathogens may return to baseline levels within days or months and the return of the pathogens to pretreatment levels generally occurs in approximately 9 to 12 weeks.[1] The reduction in the plaque index in both the SRP and the SRP+aPDT group reveals that all the patients have maintained a good oral hygiene practice.

The results of this study implied that both treatment modalities lead to statistically significant improvements in all investigated clinical parameters at 3 months following therapy. The positive clinical outcomes obtained in the SRP group are in agreement with the previously reported findings of Badersten et al (1987)[43] and Weijden et al (2002)[44] on the clinical efficacy of subgingival debridement in the treatment of chronic periodontitis that showed subgingival debridement in conjunction with supragingival plaque control was effective in reducing PD and improving CAL in subjects with chronic periodontitis.. This study is another testament for the importance of nonsurgical periodontal therapy by means of SRP.

Gingival bleeding on probing is one of the earliest signs of gingival inflammation. It implies an inflammatory lesion in the epithelium and in the connective tissue and it is is widely used to measure the prevalence and progression of the periodontal diseases, to evaluate the outcomes of treatment, and also to motivate patients with their oral care. In this study we have evaluated the % of bleeding sites before and after the interventions.[1]

At 3 months, though both the interventions yielded a positive outcome, the sites treated with SRP+aPDT resulted in a statistically significant reduction in the % of bleeding sites compared to the sites treated with SRP alone. These findings are in agreement with

studies done by Christodoulides et al (2008)[18], Braun et al (2008)[19] and Betsy et al (2014)[30].

Though bleeding on probing is not a good diagnostic predictor of progressive attachment loss, its absence is an excellent predictor of periodontal stability, therefore the absence of gingival bleeding on probing is desirable and implies a low risk of future clinical attachment loss.[1]

Intergroup comparison showed similar clinical outcomes in terms of CAL and PD changes. The results of the present study are in accordance with the studies conducted by Christodoulides et al (2008)[18], Polansky et al (2009)[22] and Balata et al (2013)[28] with different concentrations of methylene blue, different wavelengths of lasers and different resident time of photosensitizers.

Contrary to our finding Braun et al (2008)[19], Sigush et al (2010)[23], Berakdar et al (2012)[26] and Betsy et al (2014)[30] reported that aPDT acts as a beneficial adjunct to SRP in the non surgical treatment and management of chronic periodontitis.

These controversial reports might be due to the difference in the modalities of treatment especially using different concentrations and resident time of the photosensitizer, different wavelengths and irradiation time of the laser and also the application skill of the clinician.

The present study has been done in chronic periodontitis patients with type II diabetes mellitus. There are relatively very few studies done in patients with diabetes mellitus and owing to the different concentrations of photosensitizers, light-application devices, and wavelengths used in the studies mentioned, it makes direct comparisons between the

techniques used very difficult. Our study is in accordance to the study conducted by Al-Zahrani et al (2009)[2] and contradictory to the study done by Ramos et al (2016)[34] done in type II diabetes patients where multiple applications of aPDT was done.

The obvious limitation of this randomized controlled split mouth clinical trial is the evaluation of clinical parameters alone. As aPDT is based on eradication of microorganisms, microbiological analysis could assess changes in periodontal pathogens. Microbial analysis of periodontal pathogens is technique sensitive and the quantitative analysis of the periodontal pathogens was not feasible to accomplish in our institution and hence our study was restricted to the evaluation of clinical parameters alone. In addition, only single episode of PDT was performed. Another limitation of this clinical trial was the use of a periodontal probe that was not pressure-calibrated to standardize probing forces.

Need of the hour is a standardized protocol in terms of concentrations of dyes, resident time of photosensitizers, energy, application time, modes of irradiation, power settings and laser types, since lack of this makes different studies hardly comparable. Further clinical trials with proper PDT protocol, large sample size and standardized clinical, biochemical and microbiological assessment must be undertaken, to determine the efficacy of adjunctive effect of PDT with SRP.

Summary and Conclusion

SUMMARY AND CONCLUSION

Following conclusions were elucidated from the results of our study.

- Monotherapy of SRP is found to be effective in the treatment of chronic periodontitis patients with type 2 diabetes mellitus.
- A combination therapy of aPDT as an adjuvant on SRP is also found to be effective in the treatment of chronic periodontitis patients with type 2 diabetes mellitus.
- Adjunctive use of aPDT on SRP provided a significantly greater reduction in the % of bleeding sites than SRP alone but failed to show any significant differences with regard to PPD and CAL.

Presence of bleeding on probing in a treated and maintained patient population is an important risk predictor for increased loss of attachment and control of bleeding implies periodontal stability. Within the limitations of the study and based on the findings obtained from our study we summarize that though application of a single episode of aPDT to SRP failed to show an improvement in terms of PPD reduction and CAL, it resulted in a significantly greater reduction in the % of bleeding sites compared to SRP alone which signifies a halt in the disease progression.

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Annexure

ANNEXURE 1

INFORMATION SHEET

We are conducting a study on 'PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS: A RANDOMIZED CONTROLLED CLINICAL TRIAL.'

The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the present study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name & Signature / Thumb impression of the patient

Name & Signature of the investigator

Date

ANNEXURE 2

INFORMED CONSENT FORM

Photodynamic Therapy As An Adjunct To Scaling And Root Planing In Chronic Periodontitis Patients With Type 2 Diabetes Mellitus: A Randomized Controlled Clinical Trial.

Name: Age/Sex Op.No: Date:

Address:

I,_____aged_____have been informed about my role in the study.

1. I agree to give my personal details like name, age, sex, address, previous dental history & the details required for the study to the best of my knowledge.
2. I will co-operate with the dentist for my intra oral examination & extra oral examination.
3. I will follow the instructions given to me by the doctor during study.
4. I permit the dentist to take photos & I accept to undergo the procedures that are required for the study.
5. If unable to participate into study for reasons unknown, I can withdraw from the study.

In my full consciousness & presence of mind, after understanding all the procedures in my own language, I am willing & give my consent to participate in this study.

Name of the patient: Name of the investigator:

Signature/Thumb impression Signature

ஆராய்ச்சி ஒப்புதல் கடிதம்.

பெயர் :- வயது/பாலினம் :- புறநோயாளி எண் :-

விலாசம் :- தேதி :-

திரு/திருமதி..... (வயது), ஆகிய நான் கீழ் காணப்படும் நிபந்தனைகளுக்கு ஒப்புதல் அளிக்கிறேன்.

1) என் பெயர், வயது, பாலினம், முகவரி, பல் சம்பந்தப்பட்ட சிகிச்சை மற்றும் என்னுடைய முழு விபரத்தினைக் கொடுக்க நான் முழு மனதுடன் ஒப்புக் கொள்கிறேன்.

2) என்னுடைய வாயின் உள்பகுதி (அல்லது) வெளிப்பகுதியை மருத்துவர் சோதனை செய்ய ஒத்துழைக்கிறேன்.

3) நான் மருத்துவர் அளிக்கும் விதிமுறைகளை தவறாமல் கடைபிடிப்பேன்.

4) மேற்கண்ட ஆராய்ச்சிக்கான என் புகைப்படம், உமிழ்நீர் மாதிரி மற்றும் பற்கள் சம்பந்தப்பட்ட எக்ஸ்ரே எடுக்கவும், ஈறு அறுவை சிகிச்சை செய்யவும் மருத்துவருக்கு ஒப்புதல் அளிக்கிறேன்.

5) நான் மேற்கண்ட ஆராய்ச்சிசியில் பங்கு பெற முடியவில்லை என்றால், ஆராய்ச்சியில் இருந்து விலகிக் கொள்வேன்.

மருத்துவரின் ஆராய்ச்சி சம்பந்தப்பட்ட விவரங்களை முழுமையாக புரிந்து கொண்டு பிறகு, என் முழுமனதுடனும் மற்றும் சுயநினைவுடனும் இந்த மருத்துவ ஆராய்ச்சியில் பங்குபெற சம்மதிக்கிறேன்.

நோயாளியின் பெயர் :- கையொப்பம் / பெருவிரல் ரேகை.

ஆராய்ச்சியாளரின் பெயர் :- கையொப்பம் :-

ANNEXURE – 3

PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS: A RANDOMIZED CONTROLLED CLINICAL TRIAL.

CLINICAL PROFORMA

Name: O.P.No:

Age/Gender: Date:

Occupation:

Address and contact no:

Chief complaints:

Medical history:

	Diabetes		Heart disease / murmur/angina
	Hypertension		Bleeding disorders
	Rheumatic fever		Asthma
	Seizures		Psychiatric care
	Long term medications		Pregnancy

Dental history:

Personal History :

Family history:

Oral hygiene measures :

Laboratory investigations :

CLINICAL PARAMETERS - AT BASELINE Date:

PLAQUE INDEX – Loe's modification (1967)

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

PI SCORE:

GINGIVAL INDEX – Loe's modification (1967)

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

GI SCORE:

PROBING DEPTH & CLINICAL ATTACHMENT LEVEL

	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
CAL																
PD																
PD																
CAL																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Signature of the guide

CLINICAL PARAMETERS - AT 3 MONTHS Date:

PLAQUE INDEX – Loe's modification (1967)

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

PI SCORE:

GINGIVAL INDEX – Loe's modification (1967)

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

GI SCORE:

PROBING DEPTH & CLINICAL ATTACHMENT LEVEL

	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
CAL																
PD																
PD																
CAL																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Signature of the guide

INSTITUTIONAL ETHICAL COMMITTEE

KSR INSTITUTE OF DENTAL SCIENCE & RESEARCH

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KSR College of Technology,
KSR Kalvi Nagar, Tiruchengode.

Member Secretary
Dr. G.S. KUMAR, MDS.,
Principal,
KSR Institute of Dental Science & Resea
KSR Kalvi Nagar, Tiruchengode.

Ref.: 091 /KSRIDSR/EC/2014 **Date : 19.12.2014**

To

Dr.Monica Ravi,
Postgraduate Student,
Dept. of Periodontics,
KSR Institute of Dental Science & Research,

*****

Your dissertational study titled "PHOTODYNAMIC THERAPY AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS – A RANDOMIZED CONTROLLED CLINICAL TRIAL" presented before the ethical committee on 17th Dec.2014 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of Member Secretary
(Dr.G.S.Kumar)
PRINCIPAL,
K.S.R. INSTITUTE OF DENTAL,
SCIENCE & RESEARCH,
K.S.R. KALVI NAGAR,
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